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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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Robert E. Hanson
Fulbright & Jaworski L.L.P.
Suite 2400
600 Congress Avenue
Austin, TX 78701

EXAMINER	
FORD, VANESSA L	
ART UNIT	PAPER NUMBER
1645	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/699,023	Applicant(s) CHEN ET AL.	
	Examiner Vanessa L. Ford	Art Unit 1645	

-- Th MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 March 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-74* is/are pending in the application.

4a) Of the above claim(s) *33-74* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-32* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *03 March 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 13 .
4) ☒ Interview Summary (PTO-413) Paper No(s). *15* .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

1. Applicant's response and submission of new formal drawings filed March 3, 2003 are acknowledged.

Objection/Rejections Withdrawn

2. In view of Applicant's response the following rejections are withdrawn:

a) Objections to the drawings due to Applicant's submission of new formal drawings.

b) Rejection under 35 U.S.C. 102(b) of claims 1-25 and 27-32, paragraph 5, pages 3-6 of the previous Office action.

c) Rejection under 35 U.S.C. 103(a) of claims 1-32, paragraph 6, pages 6-9 of the previous Office action.

New Grounds of Rejection

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Claims 1-32 rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of obtaining a gram-negative bacterium comprising a nucleic acid sequence encoding an antibody capable of binding a target ligand comprising the steps of providing a gram-negative bacterium comprising a

nucleic acid sequence encoding an antibody in soluble form, wherein said antibody is expressed in soluble form in said gram-negative bacterium and contacting said bacterium with a labeled ligand capable of diffusing into said bacterium, washing out unbound labeled ligand and selecting the bacterium based on the presence of the labeled ligand in the bacterium and wherein the labeled ligand is less than 2000 daltons and a method of obtaining a bacterium comprising a nucleic acid sequence encoding *Fusarium solani* lipase cutinase wherein the labeled ligand is capable of diffusing into said bacterium, wherein the labeled ligands are Fluorescien dibutyrate or LysoSenor Green DND-189 (LSG) and selecting the bacterium based on the presence of the labeled ligand in the bacterium does not reasonably provide enablement providing a method of obtaining a gram-negative bacterium comprising a nucleic acid sequence encoding any candidate binding protein, wherein said binding protein is expressed in soluble form in said bacterium, contacting said bacterium with any labeled ligand capable of diffusing into said bacterium and selecting said bacterium based on the presence of said labeled ligand within the bacterium. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The specification has not provided enablement for: A) a method in the absence of a wash step in the claimed method that would enable one skilled in the art to select bacterium based on the presence of the labeled ligand in the bacterium B) molecules of greater than 2000 Da that can diffuse into a gram-negative bacterium and C) the use of nucleic acids as ligands.

As to claims 1-32, drawn the antibody embodiment, the claimed method requires "...selecting said bacterium based on the presence of said labeled ligand within the bacterium, wherein said ligand and said candidate binding protein are bound in said bacterium". How would one of skill in the art distinguish between the labeled ligand bound to the candidate binding protein, verses the labeled ligand present but not bound to the protein ? There is no wash step recited in the claimed method to remove the labeled ligand bound to the candidate binding protein or labeled ligand present but not bound to the protein from the bacterium so that one of skill in the art could distinguish between the bound candidate binding protein and that which is unbound. The mere presence of labeled ligand is not a distinguishing factor.

The claimed method recites ".... that the binding protein is expressed in soluble form in said bacterium and dependent claims recite that the "labeled ligand comprises a peptide, a polypeptide, an enzyme or a nucleic acid". Dependent claims recite "... wherein said labeled ligand is further defined as comprising a molecular weight of less than about 20,000 Da", "...wherein said labeled ligand is further defined as comprising a molecular weight of less than about 5,000 Da" and "...wherein said labeled ligand is further defined as comprising a molecular weight of greater than 600Da and less than about 30,000 Da". The specification teaches that the inventors have shown that ligands of less than 2000 Da in size can diffuse in to the periplasm and such diffusion can be increased by one or more treatments of a bacterial cell thereby rendering the outer

membrane more permeable (page 15). The specification teaches that " the inventors have defined conditions that lead to the permeation of ligands into the periplasm without loss of viability or release of the expressed proteins from the cells" (page 16). The specification teaches that "as a result cells expressing binding protein can be fluorescently labeled simply by incubating with a solution of fluorescently labeled ligand (page 16). The specification teaches in Example 8, the detection of oligonucleotide probes by antibodies expressed in the *E. coli* periplasm (page 63). Example 8 shows that the modified oligonucleotides can diffuse through the outer membrane of bacteria. Example 8 teaches that digoxigenin moiety of the oligonucleotide can be recognized by scFv antibodies specific to digoxin (anti-digoxin scFv). Example 8 teaches that cells displaying the anti-digoxin scFv antibody became clearly labeled with both digoxigenin-BODIPY™ as well as with 5-A-FL. The molecular weight of digoxigenin-BODIPY™ is 485.5 Da and the molecular weight of digoxin is 780 Da, neither has a molecular weight that is near 5,000 Da much less 20,000 to 30,000 Da. The specification also teaches that fluorescent substrates can be used to specifically label *E. coli* cells displaying the relevant enzymes in their periplasm (Example 9, page 64). Example 9 teaches that the ability to discriminate cells expressing cutinase from control cells was determined using two different commercially available substrates, one of which is fluorescein dibutyrate which has a molecular weight of about 248 Da. The specification merely teaches that treatments such as hyperosmotic shock can improve labeling significantly. The specification teaches that known agents such as calcium ions alter the permeability of the outer membrane (page 16). However, the specification fails to teach that such

altered permeability provided for the "diffusion" of the labeled ligands in the claimed molecular weight range.

The specification does not teach that candidate binding proteins of a molecular weight greater than the exclusion limit of about 650 Da to about 900 Da can cross and enter into the periplasm or cytoplasm of a gram-negative bacteria cell without facilitated transport (i.e. diffusion). The specification fail to enable the use of "labeled ligands" that have a molecular weight of about 5,000 Da or about 20,000-30,000 Da.

The teachings of the prior art regarding gram-negative transport systems, exclusion limit of molecules to cross the outer membrane and inner and outer membrane permeability are cited below:

Ames *(Journal of Bioenergetics and Biomembranes, Feb., 1988, 20(1) 1-17)* teaches that bacterial periplasmic transport systems are complex, multicomponent permeases present in gram-negative bacteria. Ames teaches that a general overall structure for bacterial transport systems is that they consists of four proteins, one of which is a soluble periplasmic protein that binds the substrate and the other three are membrane bound (see the Abstract). Ames et al teach that the liganded periplasmic protein interacts with the membrane components, which presumably form a complex and which by a series of conformational changes allow the formation of an entry pathway for the substrate (see the Abstract). Ames et al teach that the cell wall proper is commonly regarded as a widely open entirely permeable layer which confers rigidity and through which nutrients diffuse readily and the cytoplasmic membrane is imperable to almost every solute unless a special transport system is provided (page 2). Ames

teaches that transport systems response to physical treatment and osmotic shock (page 2). Decad et al, *(Journal of Bacteriology, October 1976, 128(1):325-36)* teach that the permeability function cell wall of gram-negative bacteria was investigated by producing cells with an expanded periplasmic volume and incubating them with radioactive non-utilizable oligosaccharides and polysaccharides or polyethylene glycols. Decad et al teach that only disaccharides and trisaccharides could fully diffuse into the periplasm, whereas higher molecular weight saccharides were non-penetrable. Decad et al teach that the cell wall acts as a molecular sieve with an exclusion limit near 550 to 650 daltons for saccharides (see the Abstract). Nakae et al *(The Journal of Biological Chemistry, Vo. 250, No.18, September,1975)* teach that the both the outer membrane and the peptiodoglycan layer of gram-negative bacteria acts as a barrier of the molecular sieve type for the penetration of uncharged saccharides (see the Abstract). Nakae et al teach that the exclusion limit for *E. coli* and *Salmonella typhimurium* is about 900 daltons or less for saccharides which is much smaller in comparison to gram-positive bacteria which is about 100,000 daltons for *Bacillus megaterium* (page 7363). Higgins et al *(Journal of Bioenergetics and Biomembranes, Vol. 22., No.4, 1990)* teach that bacterial binding protein-dependent transport systems are the best characterized members of the superfamily of transporters which are structurally, functionally and evolutionary related to each other (see the Abstract). Higgins et al also teach that any single system is relatively specific, different systems handle very different substrates which can be inorganic ions, amino acids, sugars, large polysaccharides or even proteins (see the Abstract). Higgins et al teach that the distinction between binding

protein-dependent transport systems and other bacterial transporters is based on two criteria: a) sensitivity to cold osmotic shock and b) differential sensitivity to metabolic inhibitors. Higgins et al teach that sensitivity of binding protein-dependent transport systems to osmotic shock is due to the loss of an essential protein component of the transport system, normally located in the periplasm between the cytoplasmic (inner) and outer membranes (pages 571-572). Higgins et al teach that in addition to the periplasmic substrate-binding protein each transport system requires a distinct complex of proteins associated with the cytoplasmic-membrane and the periplasmic binding protein delivers substrate to this protein complex, which in turn mediates its translocation across the membrane (page 572).

The prior art teaches that any single binding protein dependent system is relatively specific, different systems handle very different substrates and periplasmic substrate-binding proteins of each transport system requires a distinct complex of proteins associated with the cytoplasmic-membrane. The prior art teaches that the periplasmic binding protein delivers substrate to this protein complex, which in turn mediates its translocation across the membrane. The prior art teaches that non-facilitated transfer (i.e. diffusion) of compounds across the outer membrane has an exclusion limit of about 650 to about 900 daltons. The prior art also teaches that bacterial transport systems are sensitive to osmotic shock and physical treatment which rids the transport systems of an essential protein component which is located in the periplasm. The prior art further teaches that facilitated transport such physical treatment or osmotic shock can induce permabilization of the gram-negative outer

membrane. Webster's Ninth New Collegiate Dictionary, 1990 defines "diffusion" as the process by which particles of liquids, gases or solids intermingle as the result of their spontaneous movement caused by thermal agitation in dissolved substances move from a region of higher to one of lower concentration" (page 354).

Free diffusion (spontaneous movement) into the cytosol of a bacterium using a labeled ligand cannot be achieved. Applicants have not described which ligands can be used with which transport systems? How would hydrophilic molecules (i.e. peptides, polypeptides, enzymes or nucleic acid molecules) of about 5,000-30,000 daltons cross the outer membrane and be translocated across the bacterium's hydrophobic cytoplasmic membrane without facilitated transport? How can peptides, polypeptides, enzymes or nucleic acid molecules) of greater than 2000 daltons (about 5,000-30,000 daltons) be diffused into the interior of the cell if they cannot diffuse across the outer membrane of the bacterium?

Dependent claims recite "... the method of claim 1 wherein said ligand comprises a nucleic acid." If a nucleic acid is diffused into a bacteria cell which already contains a diverse array of nucleic acid molecules, how would one skilled in the art distinguish between the nucleic acid molecules that hybridize to the nucleic acid molecules of the bacterium and a candidate binding protein that binds the labeled ligand? Nucleic acid molecules are hydrophilic molecules, how can these hydrophilic molecules cross the hydrophobic cytoplasmic membrane by mere diffusion?

As to claims 1-32, drawn to the enzyme embodiment, Applicant has not shown the use of any enzymes and labeled ligands other than *Fusarium solani* lipase cutinase and the labeled ligands are Fluorescien dibutyrate or LysoSenor Green DND-189 (LSG). The specification has not shown that any peptides, polypeptides, enzymes other then *Fusarium solani* lipase cutinase and nucleic acids can cross the outer membrane. The prior art above has taught that any single binding protein dependent system is relatively specific, different systems handle very different substrates and periplasmic substrate-binding proteins of each transport system requires a distinct complex of proteins associated with the cytoplasmic-membrane. Therefore, how can peptides, polypeptides, enzymes other than *Fusarium solani* lipase cutinase and nucleic acids cross the outer membrane? Fluorescien dibutyrate and LysoSenor Green DND-189 (LSG) are known in the art to cross the inner membrane of a bacterium. However, the prior art as cited above teaches that non-facilitated transfer (i.e. diffusion) of compounds across the outer membrane has an exclusion limit of about 650 to about 900 daltons. Applicants have not described ligands other than Fluorescien dibutyrate and LysoSenor Green DND-189 that can cross the inner membrane of the bacterium. Webster's Ninth New Collegiate Dictionary, 1990 defines "diffusion" as the process by which particles of liquids, gases or solids intermingle as the result of their spontaneous movement caused by thermal agitation in dissolved substances move from a region of higher to one of lower concentration" (page 354). Free diffusion into the cytosol of a bacterium using labeled ligands other than Fluorescien dibutyrate and LysoSenor Green DND-189 cannot necessarily be achieved. What other ligands can be used? What transport

systems are used? How can peptides, polypeptides, enzymes or nucleic acid molecules) of greater than 2000 daltons (about 5,000-30,000 daltons) be diffused into the interior of the cell if they cannot diffuse across the outer membrane of the bacterium? How would hydrophilic molecules (i.e. peptides, polypeptides, enzymes or nucleic acid molecules) of about 5,000-30,000 daltons cross the outer membrane and be translocated across the bacterium's hydrophobic cytoplasmic membrane without facilitated transport?

Factors to be considered in determining whether undue experimentation is required are set forth in In re Wands 8 USPQ2d 1400. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art and (8) the breadth of the claims.

In view of the lack of enablement for the use of method of obtaining a gram-negative bacterium comprising a nucleic acid sequence encoding any candidate binding protein, wherein said binding protein is expressed in soluble form in said bacterium, contacting said bacterium with any labeled ligand capable of diffusing into said bacterium wherein the ligand greater than 2000 Da and selecting said bacterium based on the presence of said labeled ligand within the bacterium. There is no wash step recited in the claimed method to remove the labeled ligand bound to the candidate binding protein or labeled ligand present but not bound to the protein from the bacterium so that one of skill in the art could distinguish between the bound candidate binding

protein and that which is unbound. The mere presence of labeled ligand is not a distinguishing factor. The specification has failed to enable the diffusion of any hydrophilic molecules (i.e. peptides, polypeptides, enzymes or nucleic acids greater than 2000 Da) into a gram-negative bacterium without facilitated transport (diffusion). The specification has fail to teach one skilled in the art how to distinguish between the nucleic acid molecules used as ligands which are capable of hybridizing to the nucleic acid molecules of the interior of the bacterium and the nucleic acid molecules that are apart of the bacterium. The specification has failed to teach the use of enzymes other than *Fusarium solani* lipase cutinase and the use of labeled ligands other than Fluorescien dibutyrate and LysoSenor Green DND-189 that can cross the inner membrane of the bacterium. It is determine that there are limited working examples commensurate in scope with the instant claims and there is limited guidance provided in the specification as to how to use the claimed method. The skilled artisan is forced into undue experimentation to practice (make and use) the invention as is broadly claimed.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 1-32 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: There is no wash step recited in the claimed method to remove the labeled ligand bound to the candidate binding protein or labeled ligand present but not bound to the protein from the bacterium so that one of

skill in the art could distinguish between the bound candidate binding protein and that which is unbound. The mere presence of labeled ligand is not a distinguishing factor.

5. No claims are allowed.

Conclusion

6. Any inquiry of the general nature or relating to the status of this general application should be directed to the Group receptionist whose telephone number is (703) 308–0196.

Papers relating to this application may be submitted to Technology Center 1600, Group 1640 by facsimile transmission. The faxing of such papers must conform with the notice published in the Office Gazette, 1096 OG 30 (November 15, 1989). Should applicant wish to FAX a response, the current FAX number for the Group 1600 is (703) 308-4242.

Any inquiry concerning this communication from the examiner should be directed to Vanessa L. Ford, whose telephone number is (703) 308-4735. The examiner can normally be reached on Monday – Friday from 7:30 AM to 4:00 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lynette Smith, can be reached at (703) 308–3909.

Vanessa L. Ford
Biotechnology Patent Examiner
June 12, 2003

PATRICIA A. DUFFY
PRIMARY EXAMINER